

# Bioavailability of Selenium from Meat and Broccoli as Determined by Retention and Distribution of $^{75}\text{Se}$

JOHN W. FINLEY,\*,<sup>1</sup> MICHAEL A. GRUSAK,<sup>2</sup>  
ANNA-SIGRID KECK,<sup>3</sup> AND BRIAN R. GREGOIRE<sup>1</sup>

<sup>1</sup>*United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034;* <sup>2</sup>*United States Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX; and* <sup>3</sup>*Division of Nutritional Sciences, University of Illinois, Champaign-Urbana, IL*

Received August 8, 2003; Accepted October 24, 2003

## ABSTRACT

The concentration of selenium (Se), an essential nutrient, is variable in foods, depending, in part, on how and where foods are produced; some foods accumulate substantial amounts of Se when produced on high-Se soils. The chemical form of Se also differs among foods. Broccoli is a Se-accumulating plant that contains many methylated forms of Se, and Se bioavailability from broccoli has been reported to be low. Red meats such as pork or beef could accumulate Se when the animal is fed high-Se diets, and Se from such meats has been reported to be highly bioavailable for selenoprotein synthesis. In a further attempt to characterize the utilization of Se from broccoli and meats such as pork or beef, we have fed rats diets adequate (0.1  $\mu\text{g}$  Se/g diet) in Se or high in Se (1.5  $\mu\text{g}$  S/g diet), with the Se source being either high-Se broccoli or beef. Rats were then given test meals of broccoli or pork intrinsically labeled with  $^{75}\text{Se}$ . When dietary Se was nutritionally adequate (0.1  $\mu\text{g}$ /g diet), more  $^{75}\text{Se}$  from pork than broccoli was retained in tissues; however, there were no significant differences in whole-body retention when dietary Se was high (1.5  $\mu\text{g}$ /g diet). A significantly greater percentage of  $^{75}\text{Se}$  from broccoli than pork was excreted in the urine and dietary Se did not affect urinary excretion of broccoli  $^{75}\text{Se}$ , but the amount excreted from pork varied directly with dietary Se intake.

\* Author to whom all correspondence and reprint requests should be addressed.

Radiolabeled  $^{75}\text{Se}$  derived from pork effectively labeled selenoproteins in all tissues examined, but  $^{75}\text{Se}$  from broccoli was undetectable in selenoproteins. These differences in retention and distribution of Se from broccoli or pork are consistent with reported differences in bioavailability of Se from beef and broccoli. They also suggest that there are fewer differences in bioavailability when Se is consumed in supranutritional amounts.

**Index Entries:** Selenium; bioavailability; broccoli; pork; beef; selenoprotein.

## INTRODUCTION

Selenium (Se), an essential nutrient, has many biological functions, such as an antioxidant (1) and enhancement of immune function (2). The best known health benefit of supplemental Se is its function in suppressing the incidence and mortality of several important cancers (3). Selenium metabolism (and physiological effects of Se) is primarily determined by the organic molecule to which it is bound (4,5). Selenomethionine (SeMet), the Se analog of methionine, is a common form in many foods that randomly substitutes for methionine during protein production and accumulates in large protein masses such as the muscle (6). As the protein pool turns over, the SeMet may be released and moved into other pools. Inorganic forms of Se such as selenate and selenite are nonenzymatically metabolized to selenide, which could move into the urinary excretory pathway or be incorporated as selenocysteine (SeCys) into specific selenoproteins {e.g., thioredoxin reductase [TR] or glutathione peroxidase [GSH-Px] (7–9)}. Methylated selenomolecules move readily into the excretory pathway, where they may produce metabolites that some studies suggest are primary anticarcinogens (10). Thus, the chemical form of Se could predetermine its metabolic fate and, perhaps, the eventual physiological action.

The American Dietetic Association urges that, as much as possible, people should consume nutrients through whole foods (11) and not as specific supplements. However, because foods differ in their forms of Se, it is possible that different foods will induce different physiological outcomes. Aside from studies that have demonstrated the ability of wheat and meat sources of Se to increase blood Se concentrations and/or GSH-Px activities (12–15) and reports of the cancer-protective properties of Se-enriched broccoli (16), garlic (17), onions, and Brazil nuts (18), there have been few reports of the metabolism of foods enriched in Se. Studies of Se in food are complicated because the food matrix and Se could interact in unpredictable ways and because most foods are a complex mixture of multiple forms of Se (19).

Broccoli is unique because it might sequester high concentrations of Se in the edible inflorescence ( $>500 \mu\text{g Se/g}$  dry broccoli powder) (20).

Based on the ability to induce tissue accumulation of Se and/or GSH-Px activity, Se from broccoli has relatively low bioavailability (21). A limited number of studies in animal models have suggested that Se from high-Se broccoli is effective for reducing preneoplastic lesions associated with colon cancer (16,22–24).

The most abundant single food source of Se in the North American diet is beef (25). Most Se in beef is probably in the form of SeMet or SeCys, although this might depend on the feedstuffs consumed by the animal (26). Selenium from beef is highly bioavailable to rats when based on tissue Se accumulation and induction of GSH-Px activity (15,27). Van der Torre et al. (13) reported that Se from beef was highly bioavailable to humans.

The objective of the present study was to further characterize differences in the utilization (bioavailability) and metabolism of Se from red meats and broccoli by studying the retention and distribution of  $^{75}\text{Se}$  intrinsically incorporated into the food. The retention of  $^{75}\text{Se}$  was studied in the whole body, individual tissues and organs, and specific proteins.

## MATERIAL AND METHODS

### *High-Se Broccoli and Beef*

High-Se broccoli was produced as previously described (16) by adding a liquid solution of sodium selenate to broccoli planted in a soilless mixture. At maturity, the edible inflorescence was harvested, lyophilized, ground to powder, and added to animal diets. High-Se beef was produced as previously reported (28). Briefly, young steers were fed for 105 d a diet that contained Se-enriched alfalfa hay and wheat. Animals were slaughtered and lean meat was ground into burger. The burger was thoroughly cooked, drained, lyophilized, ground to powder, and included in the diet. Low-Se broccoli was purchased from a local grocery store; low-Se beef came from a herd in central Virginia that did not receive supplemental Se.

### *$^{75}\text{Se}$ -Labeled Broccoli and Pork*

A 3-wk-old crossbred female pig fed a Se-deficient torula yeast diet (4.68 ng Se/g diet) was orally dosed with 2.5 mCi carrier-free  $^{75}\text{Se}$  as selenite (University of Missouri–Columbia Research Reactor Center, Columbia, MO) over a 3-d period. After 6 d, the pig was euthanized and all skeletal muscle was removed, flash frozen, lyophilized, and ground to a powder.

Broccoli plants (cultivar “Emperor,” F<sub>1</sub> hybrid) were grown hydroponically in an environmental growth chamber (Convion Model PGW36; Winnipeg, Manitoba, Canada). All plants were maintained on a 16-h, 20°C/8-h, 15°C day–night regime and 70% relative humidity. Light was provided during the day from a combination of fluorescent and incandescent lamps; the intensity of photosynthetically active radiation

was 500  $\mu\text{mol photons/m}^2/\text{s}$  at the top of mature plants. Plants were started as previously described (29) and then were grown hydroponically (two plants per container) in 20 L of nutrient solution containing the following macronutrients (in mM):  $\text{KNO}_3$ , 0.5;  $\text{Ca}(\text{NO}_3)_2$ , 0.5;  $\text{KH}_2\text{PO}_4$ , 0.25;  $\text{MgSO}_4$ , 0.25; and the following micronutrients (in  $\mu\text{M}$ ):  $\text{CaCl}_2$ , 25;  $\text{H}_3\text{BO}_3$ , 25;  $\text{MnSO}_4$ , 2;  $\text{ZnSO}_4$ , 0.2;  $\text{CuSO}_4$ , 0.5;  $\text{H}_2\text{MoO}_4$ , 0.5;  $\text{NiSO}_4$ , 0.1. Iron was added in chelated form as  $\text{Fe}(\text{III})$ -EDDHA (*N,N'*-ethylene bis[2-(2-hydroxyphenyl)-glycine]) at 5  $\mu\text{M}$ . 2-Morpholinoethane sulfonic acid (MES) buffer (adjusted with KOH) was added at 2 mM to maintain nutrient solution pH between 5.4 and 5.8. Solutions were changed weekly during the first 10 wk of growth.

After 11 wk, the plants were provided fresh nutrient solution of the same composition, but with the addition of approx 2 mCi of  $^{75}\text{Se}$  (as carrier-free sodium selenite, added to the total nutrient solution for both plants). The plants were maintained on this solution for an additional 10 d, during which time they absorbed all of the nutrient solution. Broccoli heads were then harvested, frozen, lyophilized, and ground to a powder.

### **Experiment I: Animals and Diets**

Thirty-eight male rats (weanling Sprague-Dawley) were divided into three groups and fed a torula yeast Se-deficient basal diet (Harlan Tek-Lad, Madison, WI). Ten rats were fed the basal diet with high-Se beef added in sufficient amounts to give a final dietary Se concentration of 1.5  $\mu\text{g Se/g diet}$ ; 10 rats were fed the basal diet plus a sufficient amount of high-Se broccoli to give a final Se concentration of 1.5  $\mu\text{g Se/g diet}$ . Eighteen rats were fed the Se-deficient torula yeast basal diet without added Se. All diets were equalized for total beef and broccoli content by adding low-Se broccoli or beef in the same proportions as the high-Se diets; thus, all diets contained 0.17% broccoli and 22.7% beef. Low-Se beef and broccoli caused the low-Se diets to be approx 0.1  $\mu\text{g Se/g diet}$ ; thus, they were not deficient, but rather adequate in Se. After 6 wk, two rats from each group were killed to determine Se status (GSH-Px activity and Se concentration in selected tissues).

After 6 wk of diet, rats were fed test meals of  $^{75}\text{Se}$ -labeled pork or broccoli. All test meals contained the same amount of radioactivity (0.5  $\mu\text{Ci Se}$ ) regardless of source. To facilitate consumption, radiolabeled pork and broccoli were mixed with the low-Se diet to make a total test meal of 3 g (pork test meals contained approx 1.7 g of  $^{75}\text{Se}$ -labeled pork, and broccoli test meals contained approx 0.46 g of  $^{75}\text{Se}$ -labeled broccoli) with a total Se content of less than 2.0  $\mu\text{g}$ . Animals on the high-Se broccoli diet were fed the broccoli test meal and animals on the high-Se beef diets were fed the pork test meal. In the group fed the basal diet, one-half of the animals were fed test meals containing 0.5  $\mu\text{Ci } ^{75}\text{Se}$  as pork and the others were given 0.5  $\mu\text{Ci } ^{75}\text{Se}$  as broccoli. Following the test meals, rats were

placed in metabolic cages and given their respective diets. Rats, urine, and feces were collected and counted daily with a custom-built Small Animal Whole Body Gamma Counter consisting of a counting chamber between two sodium iodide detectors (30). Rats were killed 2 or 10 d after the test meal, and organs and blood were removed and counted in a gamma well counter (Packard Cobra II gamma counter; Packard Instrument Co., Downers Grove, IL).

### ***Experiment II: Incorporation of $^{75}\text{Se}$ from Pork or Broccoli into Selenoproteins***

Eight Fisher 344 male weanling rats (Charles River/Sasco, Wilmington, MA) were fed a Se-deficient torula yeast diet for 6 wk. Animals were divided into two groups and fed daily for 11 d test meals that contained 1  $\mu\text{Ci}$   $^{75}\text{Se}$  as labeled pork or broccoli (total of 11  $\mu\text{Ci}$   $^{75}\text{Se}$ /rat). After 11 d, rats were returned to the torula yeast diet for 6 additional days, then killed and tissues/organs collected.

Rat tissues were homogenized (Pro 250 tissue homogenizer; Pro Scientific, Monroe, CT) 3X 5 s in 20 mM Tris buffer containing 1 mM EGTA, 5 mM EDTA, 25 mM sucrose, and protease inhibitor cocktail for mammalian tissue (Sigma, St. Louis, MO). The homogenate was centrifuged at 1800g for 15 min and then the supernatant was removed and centrifuged at 100,000g for 1 h. The protein concentration was determined using the Bio-Rad method (Bio-Rad, Hercules, CA). Proteins (50  $\mu\text{g}$ /lane) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 4–20% Tris-glycine gels (Invitrogen, Carlsbad, CA), transferred to polyvinylidene difluoride (PVDF) membranes (Laemmli buffer with 0.01% SDS and 20% methanol at 30 V for 2 h), stained, dried, and placed on a phosphor storage screen for 5 d. The screen was read using a Storm 860 Phosphor imager (Molecular Dynamics, Sunnyvale, CA). Bands were compared using Imagequant software (Molecular Dynamics). Molecular weights were determined by comparing to SeeBlue Plus 2 prestained protein standard (Invitrogen, Carlsbad, CA).

### ***Statistics***

Turnover rates of  $^{75}\text{Se}$  were estimated by the two-component exponential model: % retention =  $b_1e^{-k_1t} + b_2e^{-k_2t}$ , where  $b_1$  and  $b_2$  represent the percent of  $^{75}\text{Se}$  turning over at rates  $k_1$  and  $k_2$ , respectively, and  $t$  is time (in days). The biological half-life,  $t_{1/2}$ , for each component was estimated using the formula  $t_{1/2} = \ln(2)/k_i$ ,  $i = 1, 2$ .

Retention estimates for whole-body, urine, feces, and organs were statistically analyzed by 2x2 analysis of variance (ANOVA), with the amount of dietary Se and source of Se as independent factors. Tukey's contrasts compared individual means when the dietary Se  $\times$  source interaction was statistically significant ( $p < 0.05$ ). Data are reported as mean  $\pm$  SE.

## RESULTS

### *Experiment I*

#### *Selenium Status*

As determined in rats killed before the test meal, high-Se broccoli and beef diets increased Se status compared to Se-adequate diets. Liver cytosol Se concentrations in rats fed 0.1  $\mu\text{g}$  Se/g diet were  $6.3 \pm 0.2$   $\mu\text{g}$  Se/g protein and they increased to  $12.4 \pm 1.4$   $\mu\text{g}$  Se/g protein in rats fed high-Se diets. Whole-blood GSH-Px was  $193.1 \pm 12.0$  mU/mg Hb in rats fed adequate Se and  $321 \pm 5.2$  mU/mg Hb in animals fed high-Se diets. There were no significant differences in either food intake or weight gain for any of the treatment groups.

#### *Whole-Body $^{75}\text{Se}$ Retention and $^{75}\text{Se}$ Excretion in Urine and Feces*

A two-component exponential model provided an excellent fit to whole-body retention of  $^{75}\text{Se}$  of all rats (see Fig. 1A,B). The biological half-life ranged from 0.4 to 1.1 d for the first component and from 4.1 to 41.6 d for the second component (see Table 1). The majority of broccoli  $^{75}\text{Se}$  (68–73%) was in the first component; pork  $^{75}\text{Se}$  was primarily in the first exponential component when dietary Se was high (76%), but in the second component when dietary Se was adequate (75%). When dietary Se was high, the biological half-life of  $^{75}\text{Se}$  from pork and broccoli were similar, but when it was adequate, the biological half-life of Se from pork was always longer than half-life of Se from broccoli. This occurred in both the first and model components.

Absorption of  $^{75}\text{Se}$  (total  $^{75}\text{Se}$  – total fecal  $^{75}\text{Se}$  d 1–4) was 75–85% for all sources (Table 1). More broccoli  $^{75}\text{Se}$  was excreted in the urine than pork  $^{75}\text{Se}$  (Table 1), and urinary  $^{75}\text{Se}$  excretion was greater with the high-Se diet than the Se-adequate diet. Rats fed the high-Se diet excreted 47% more  $^{75}\text{Se}$  from broccoli into urine than rats fed the adequate-Se diet. However, rats fed the high-Se diet and  $^{75}\text{Se}$  as pork excreted 282% more  $^{75}\text{Se}$  into urine than rats fed the adequate-Se diet and pork.

#### *Retention of $^{75}\text{Se}$*

The percent retention of  $^{75}\text{Se}$  from pork or broccoli in the liver, kidney, testes, plasma, erythrocytes, vesicular gland, heart, lung, spleen, muscle, brain, thymus, prostate, adrenal, pituitary, and epididymis 2 and 10 d following consumption of the labeled test meal are shown in Fig. 2A–H. Rats fed adequate dietary Se retained pork  $^{75}\text{Se}$  better than broccoli  $^{75}\text{Se}$  in all organs/tissues except the prostate and brain (d 2), vesicular gland and pituitary (d 2 and 10) and erythrocytes (d 10). The greatest difference in retention of pork and broccoli  $^{75}\text{Se}$  in rats fed Se-adequate diets was in the plasma (pork  $^{75}\text{Se}$  retention fourfold > broccoli  $^{75}\text{Se}$  retention)



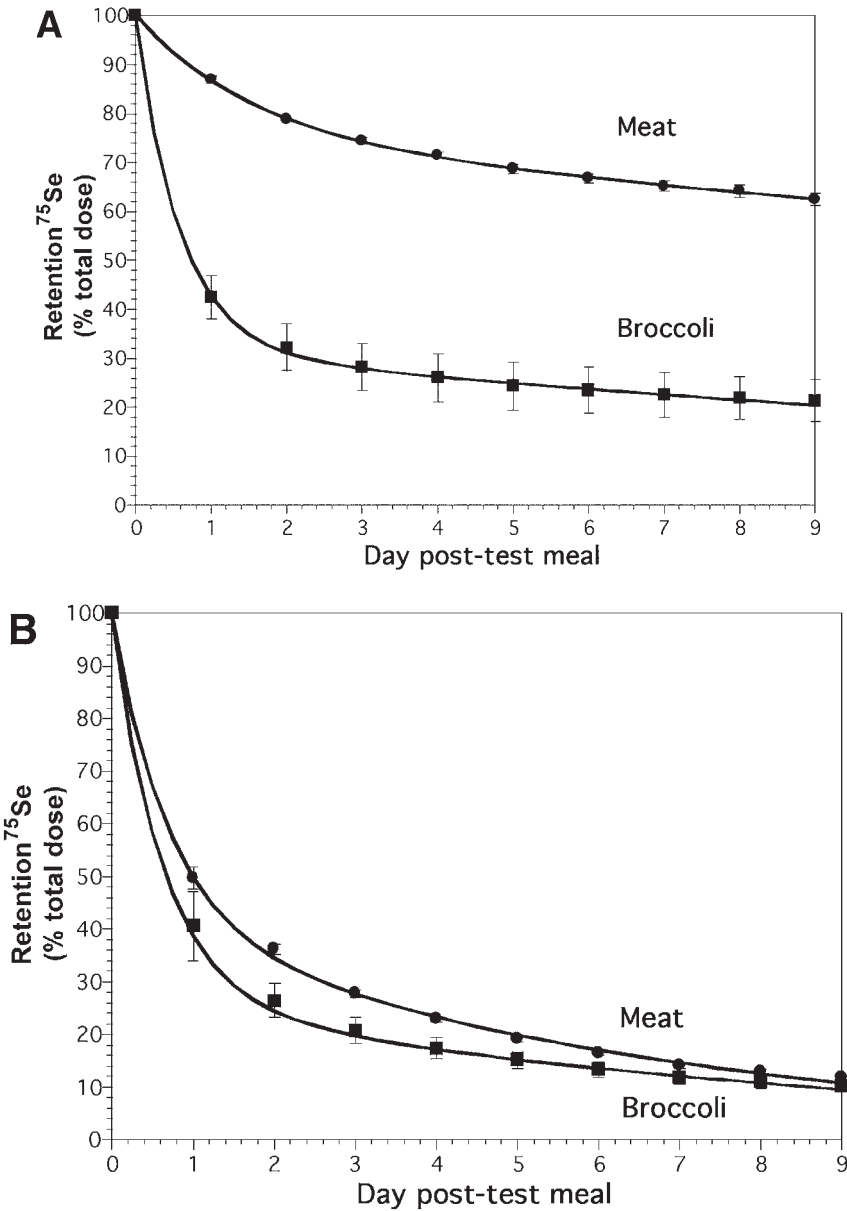


Fig. 1. Whole-body retention of  $^{75}\text{Se}$  in male Sprague-Dawley rats fed a 0.1- $\mu\text{g}$  Se/g torula yeast basal diet (**A**) containing either normal broccoli ( $\blacksquare$ ,  $n=3$ ) or beef ( $\bullet$ ,  $n=3$ ); or a .5- $\mu\text{g}$  Se/g diet torula yeast-based diet (**B**) with Se supplied as either high-Se broccoli ( $\blacksquare$ ,  $n=3$ ) or beef ( $\bullet$ ,  $n=3$ ). After 6 wk, each animal was fed a test meal containing 0.5  $\mu\text{Ci}$   $^{75}\text{Se}$  as labeled pork or broccoli. Animals fed the broccoli-supplemented diet were fed the broccoli test meal and animals fed the beef-supplemented diet were fed the pork test meal. Following test meals, rats were placed in metabolic cages and given their respective diets for the remainder of the study. Urine and feces were collected and counted daily on a custom-built Small Animal Whole Body Gamma Counter consisting of a counting chamber between two sodium iodide detectors.

Table 1  
Effect of Source and Amount of Dietary Se on Retention of <sup>75</sup>Se in the Whole Body and Excretion of <sup>75</sup>Se into Urine and Feces

Se concentration (µg/g diet)	0.1		1.5		ANOVA	
Se source	Broccoli	Pork	Broccoli	Pork	Se	SeXsource
Absorption, %	79.0±1.3 <sup>ab</sup>	82.0±0.5 <sup>a</sup>	80.4±0.6 <sup>ab</sup>	75.8±1.6 <sup>b</sup>	NS <sup>2</sup>	0.009
<b>1st Component</b>						
% in 1 <sup>st</sup> component	68.1±5.2 <sup>bc</sup>	24.9±1.2 <sup>a</sup>	72.9±1.4 <sup>c</sup>	57.2±0.6 <sup>b</sup>	0.0002	0.0001
ln (T <sub>1/2</sub> 1 <sup>st</sup> component) <sup>3</sup>	-0.89±0.06 <sup>a</sup>	0.08±0.05 <sup>b</sup>	-0.78±0.24 <sup>a</sup>	-0.73±0.12 <sup>a</sup>	0.03	0.006
mean.d	0.41	1.09	0.46	0.48		
range, d	(0.36-0.45)	(1.01-1.19)	(0.32-0.72)	(0.3843-0.54)		
<b>2nd component</b>						
% in 2 <sup>nd</sup> component	31.9±5.2 <sup>ab</sup>	75.1±1.0 <sup>c</sup>	27.1±1.4 <sup>a</sup>	42.8±0.6 <sup>b</sup>	0.0002	0.0001
ln (T <sub>1/2</sub> 2 <sup>nd</sup> component)	2.7±0.1 <sup>b</sup>	3.5±0.2 <sup>c</sup>	1.8±0.1 <sup>a</sup>	1.5±0.04 <sup>a</sup>		
mean	14.0	34.5	6.0	4.5	0.0001	0.001
range	(13.3-16.6)	(33.3-47.4)	(5.6-6.8)	(4.4-4.8)		
Urinary excretion, % dose	55.5±4.2 <sup>b</sup>	16.4±0.3 <sup>a</sup>	81.2±4.9 <sup>c</sup>	62.7±4.4 <sup>b</sup>	0.0001	0.0001
						0.03

Note: Rats fed Se-adequate diets (0.1 µg Se/g diet) or high-Se diets (1.5 µg Se/g diet) were fed a test meal of <sup>75</sup>Se-labeled meat or broccoli; they were placed in metabolic cages and counted in a whole-body counter for 10 d. Data are expressed as the mean ± SE (n=3) or mean and range. Different superscripts denote means that are significantly different (p<0.05).

<sup>1</sup> Absorption = 100% - fecal retention from the first 4 d.

<sup>2</sup> NS = none significant.

<sup>3</sup> t<sub>1/2</sub> = biological half-life.



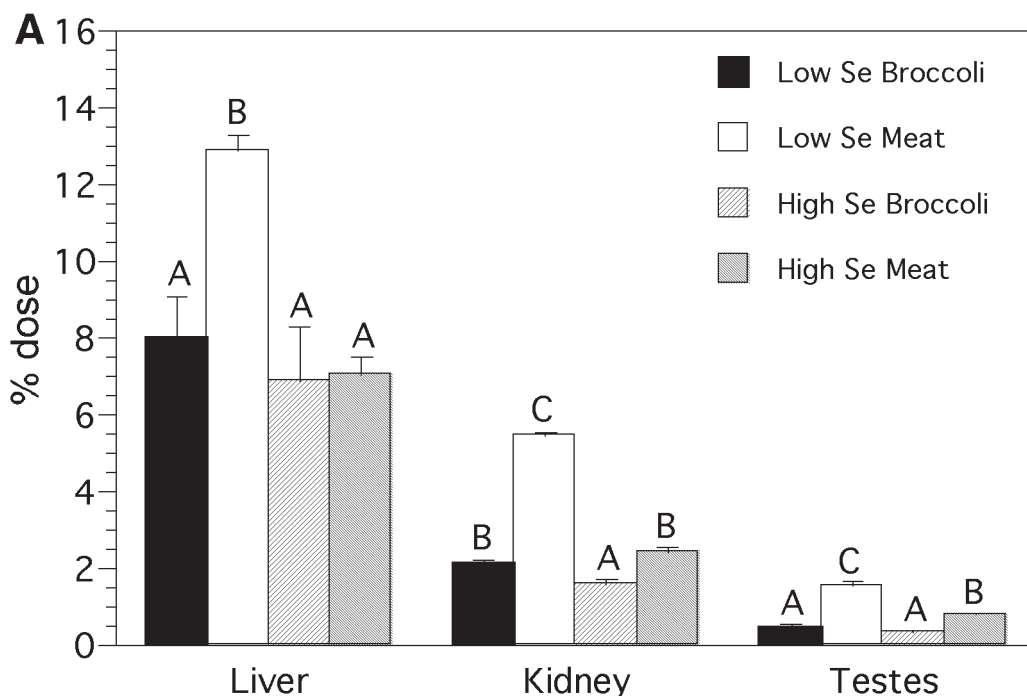


Fig. 2. Retention of  $^{75}\text{Se}$  in rats fed either an adequate-Se diet ( $0.1 \mu\text{g Se/g}$  diet) or a diet containing  $1.5 \mu\text{g Se/g}$  diet as high-Se broccoli or high-Se beef and then fed a test meal containing  $^{75}\text{Se}$  as labeled Se in broccoli or pork. Retention is shown either 2 or 10 d after consumption of the test meal. Retention of  $^{75}\text{Se}$  is shown for liver, kidney, and testes 2 d (A) or 10 d (B) after consumption of the test meal; for plasma, red blood cells (RBCs) or vesicular gland 2 d (C) or 10 d (D) after consumption of the test meal; for heart, lung, spleen, muscle, brain, and thymus 2 d (E) or 10 d (F) after the test meal; for prostate, adrenal, pituitary, and epididymis 2 d (G) or 10 d (H) after consumption of the test meal. Data are expressed as the mean  $\pm$  SE percentage of total ingested counts per organ or per gram tissue. Contrasts were computed whenever the interaction of dietary Se concentration  $\times$  source of  $^{75}\text{Se}$  was significant ( $p < 0.05$ ). Different superscripts denote means that are significantly different. <sup>1</sup>No significant effects of dietary Se concentration, source of  $^{75}\text{Se}$ , or interaction ( $p > 0.05$ ). <sup>2</sup>No interaction of dietary Se concentration  $\times$  source of  $^{75}\text{Se}$ ; significant effect of dietary Se concentration ( $p < 0.05$ ). <sup>3</sup>No interaction of dietary Se concentration  $\times$  source of  $^{75}\text{Se}$ ; significant effect of source of  $^{75}\text{Se}$  ( $p < 0.05$ ). No superscript = significant interaction of dietary Se concentration  $\times$  source of  $^{75}\text{Se}$  ( $p < 0.05$ ). (Figure continues)

followed by kidney, testes, thymus, spleen, adrenal > lung, heart, epididymis, liver > muscle. In contrast, when rats were fed high-Se diets,  $^{75}\text{Se}$  from pork or broccoli was retained equally well on d 2 in all tissues/organs except the thymus, brain, lung, plasma, and testes and in all tissues/organs on d 10.

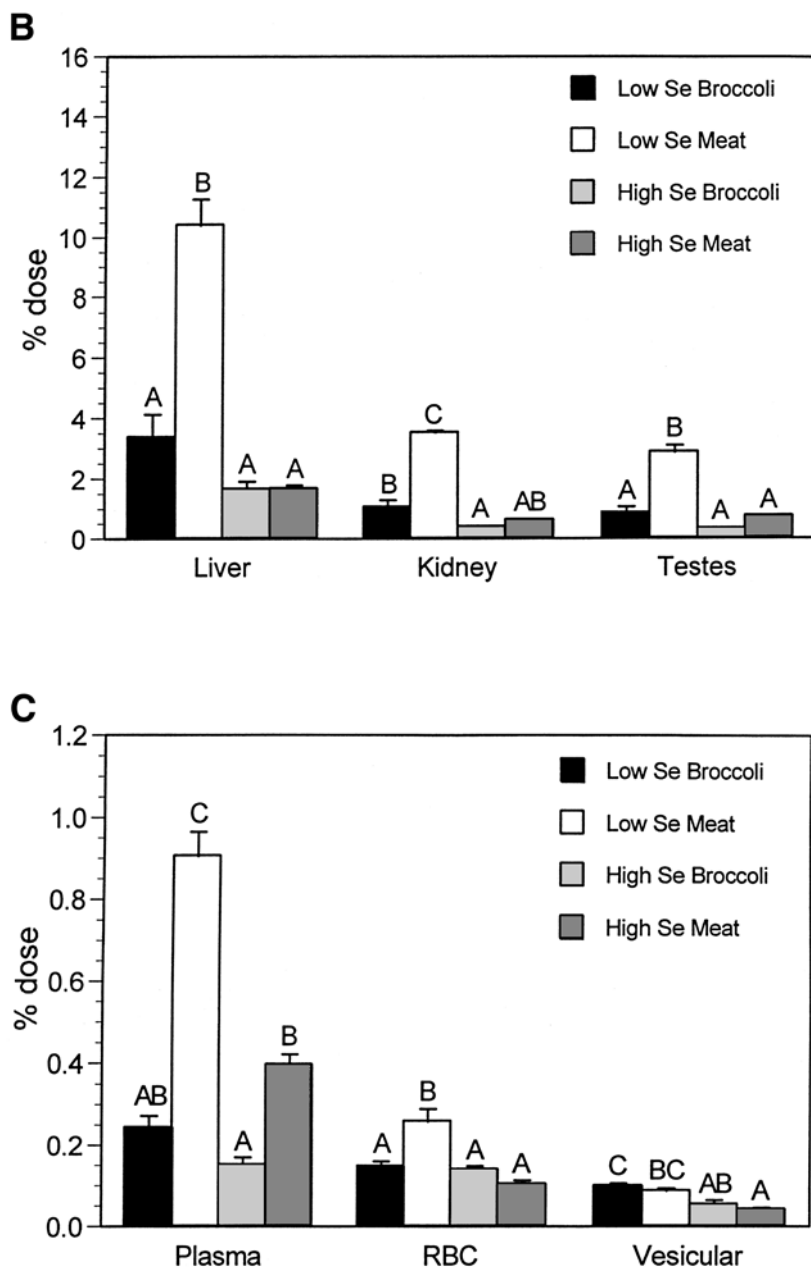


Fig. 2. (Continued)

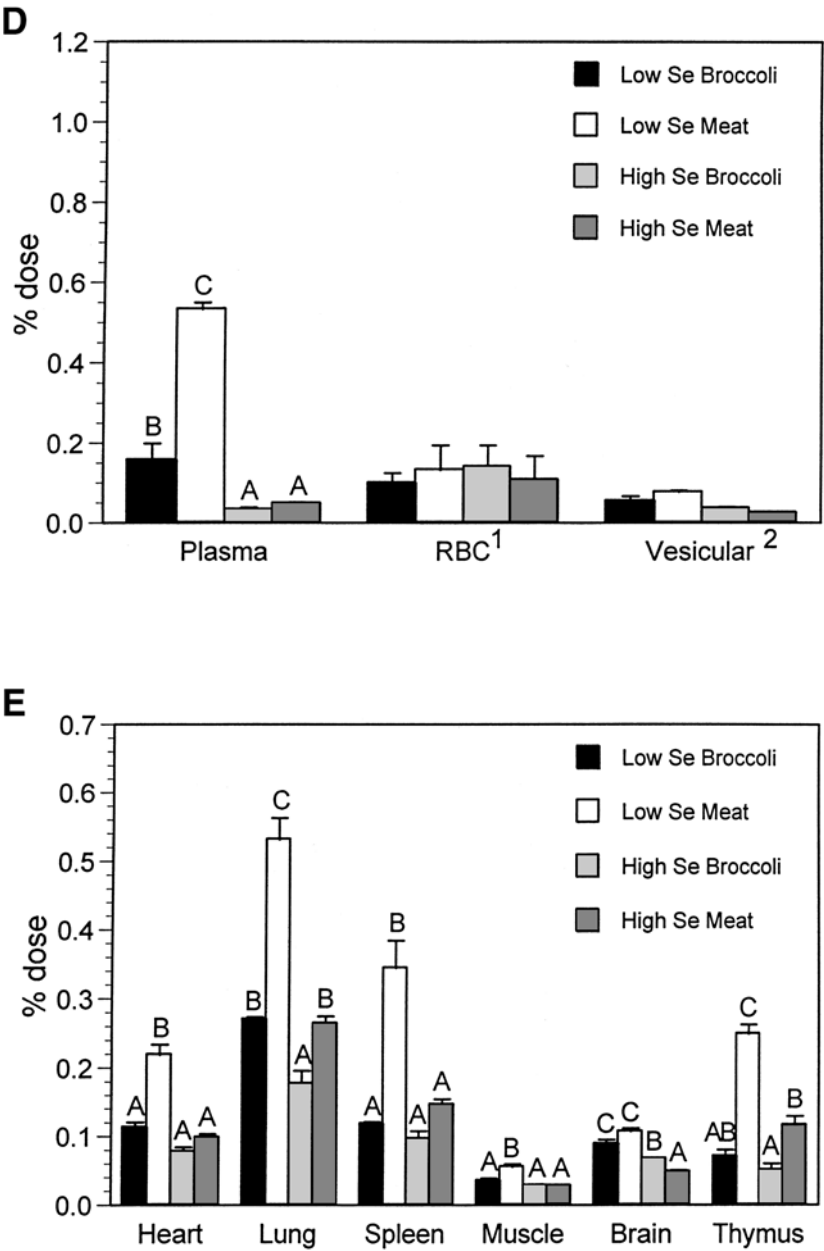


Fig. 2. (Continued)

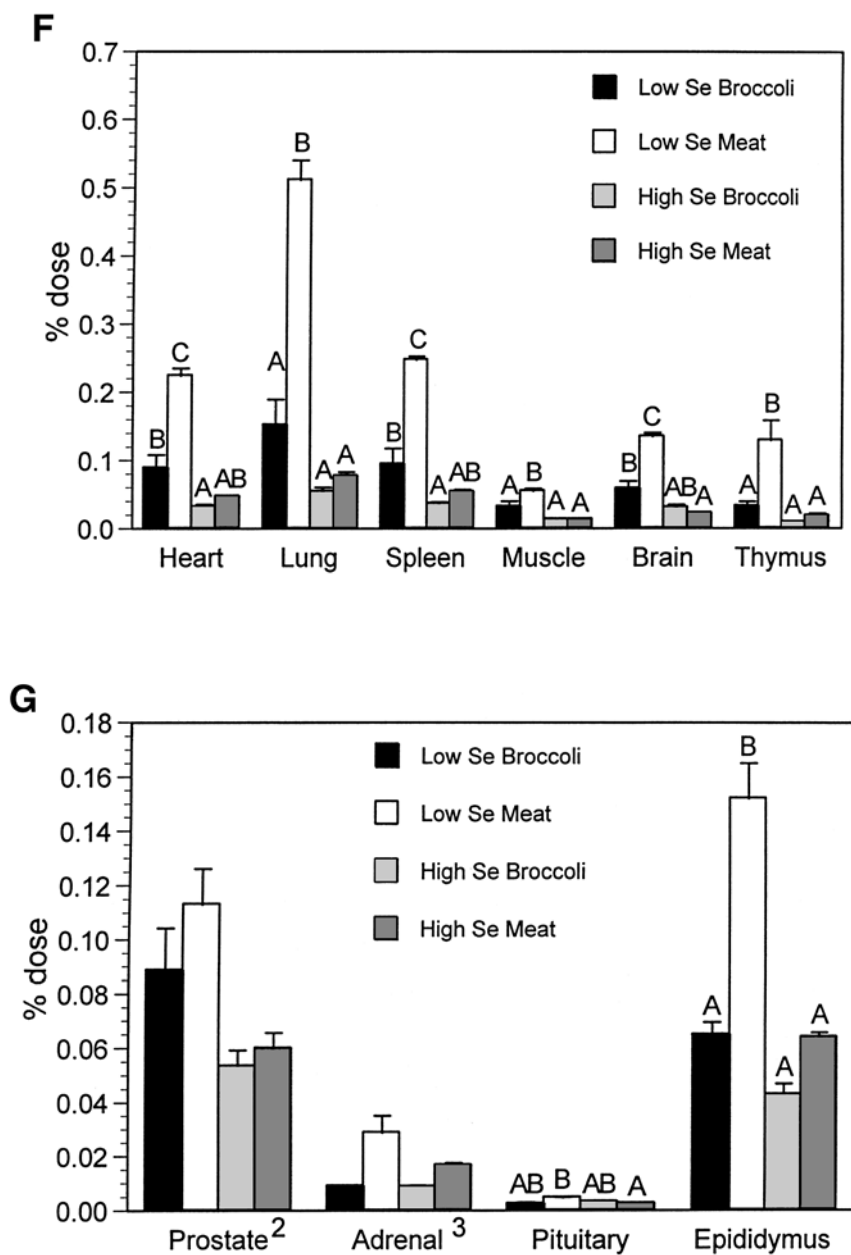


Fig. 2. (Continued)

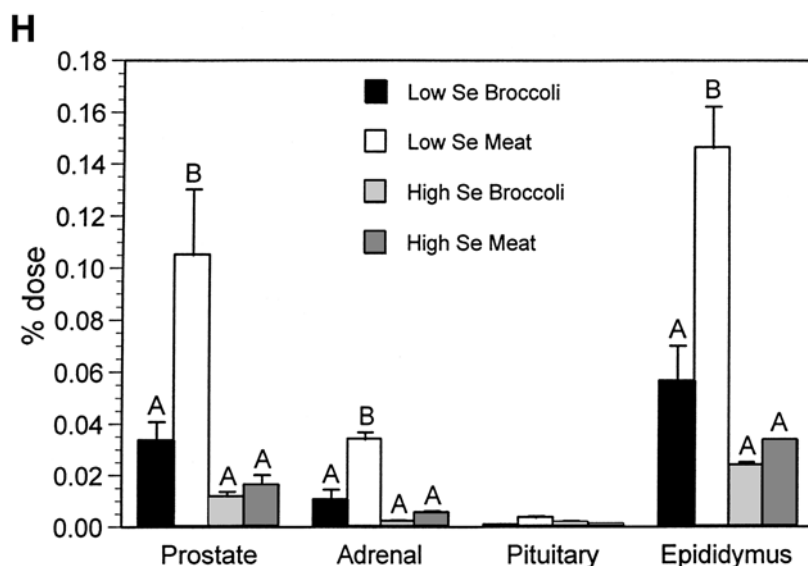


Fig. 2. (Continued)

When compared within a source (i.e., broccoli or pork), the amount of dietary Se, in most tissues, significantly affected retention of  $^{75}\text{Se}$  from pork but not broccoli. In rats given pork  $^{75}\text{Se}$ ,  $^{75}\text{Se}$  retention was significantly higher (twofold to fourfold higher depending on tissue) in rats fed high, as compared to adequate, dietary Se. However, retention of broccoli  $^{75}\text{Se}$  was only affected by dietary Se in the kidney, vesicular gland, lung, and brain on d 2 and in kidney, plasma, heart, and spleen on d 10 (high-Se diet resulted in lower retention of  $^{75}\text{Se}$  in these tissues).

Retention of  $^{75}\text{Se}$  decreased between d 2 and d 10 across all treatments in the thymus, kidney, plasma, and spleen. In most other tissues,  $^{75}\text{Se}$  from broccoli disappeared more rapidly (i.e., retention on d 2 > d 10) than  $^{75}\text{Se}$  from pork. Pork  $^{75}\text{Se}$  disappeared much faster in rats fed high-Se, as compared to Se-adequate diets. In fact, in rats fed adequate Se, there was little or no decrease in pork  $^{75}\text{Se}$  in the prostate, heart, lung, brain, and liver.

## Experiment II

The  $^{75}\text{Se}$  from pork was well incorporated into proteins as determined by SDS-PAGE (Fig. 3), but an equal amount of  $^{75}\text{Se}$  supplied as broccoli resulted in almost no incorporation into proteins.

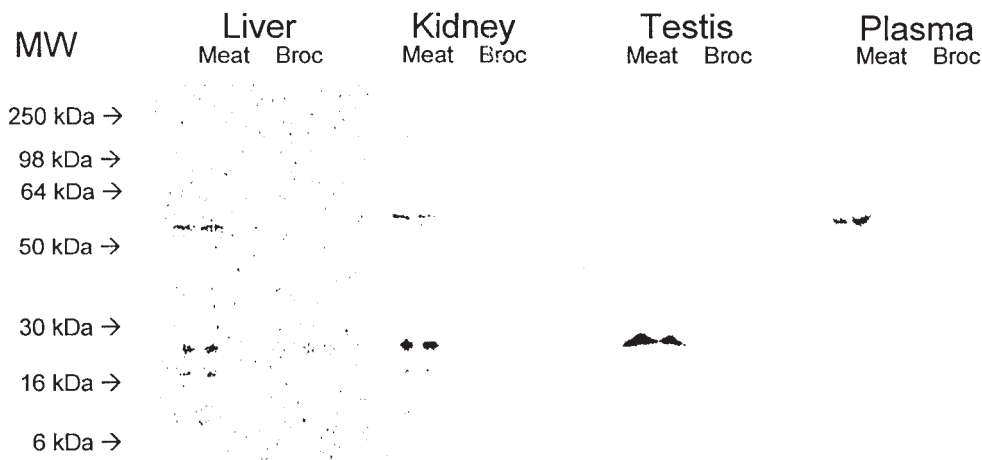


Fig. 3. Incorporation of  $^{75}\text{Se}$  from broccoli or pork into liver, kidney, testes, and plasma selenoproteins as determined by SDS-PAGE. Eight Fisher 344 male rats were fed a Se-deficient torula yeast diet for 6 wk. Animals were divided into two groups and fed daily for 11 d test meals that contained 1  $\mu\text{Ci}$   $^{75}\text{Se}$  as labeled pork or broccoli (total of 11  $\mu\text{Ci}$   $^{75}\text{Se}$ /rat). After 11 d, rats were returned to the torula yeast diet for 6 additional days, then killed and tissues/organs collected. Proteins were separated by SDS-PAGE gels and transferred to a PVDF membrane; membranes were stained, dried, and read using a Storm 860 phosphor imager.

## DISCUSSION

Animal and human data provide a compelling argument for augmenting dietary Se intakes. However, foods contain variable amounts of Se (31). If foods are to be recommended as sources of supplemental Se, they need to be produced in a way that ensures a certain Se content, and the Se must be able to be utilized by the body. Previous studies have relied on repletion of GSH-Px activity and tissue Se concentrations to estimate Se bioavailability. The present report has utilized  $^{75}\text{Se}$ -labeled broccoli and pork to more fully characterize the biological utilization of Se from these foods. Results show that Se from meat and broccoli enter different kinetic pools, have different tissue retention profiles, and do not have the same efficacy for incorporating into selenoproteins. These differences account for much of the reported differences in the bioavailability of Se from beef and broccoli.

Because the food matrix can alter nutrient metabolism and because Se is covalently incorporated into selenomolecules in food, the present study has utilized broccoli and pork radiolabeled by adding  $^{75}\text{Se}$  to the growing plant and animal (32). Our diets included beef and radiolabeled beef was the ideal tracer, but production of radiolabeled beef (and disposal of the large amounts of radioactive waste) is impractical and a young pig was substituted. This is a valid substitution because lean pork and beef are sim-

ilar in composition (33–36), Se bioavailability (37), lean meat amino acid profile (33), and nutrient contribution to the US diet (except for thiamine, folate, and Mg) (34). When prepared by the same method, the nutritional composition of processed pork and beef carcasses are almost identical (35) and, except for iron and vitamin B<sub>12</sub>, have almost identical proximate analyses and mineral and vitamin composition (36).

As suggested by Martin et al. (38), a two-component exponential model with a fast (labile) and slow turnover (stable) pool of Se provided an excellent fit to whole-body radiation data. Some of the fast-turnover pool of <sup>75</sup>Se was gut passage of unabsorbed Se because 25% of pork <sup>75</sup>Se in rats fed adequate Se was calculated to be in the fast-turnover pool, which approximated the 18% of unabsorbed pork <sup>75</sup>Se determined by fecal balance. However, unabsorbed Se did not account for the fast-turnover pool in other treatments; 21% of broccoli <sup>75</sup>Se was unabsorbed by rats fed adequate Se, but 68% was calculated to be in the fast compartment. The kinetics of urinary excretion, the primary means of maintaining Se homeostasis, also are biphasic with a short- and long-term component (39); thus, the fast-turnover compartment is probably a mixture of unabsorbed Se in luminal transit as well as urinary excretion.

Depending on Se status, <sup>75</sup>Se from broccoli and pork went into different pools, and this was associated with the amount excreted in the urine. The majority of broccoli <sup>75</sup>Se was in the fast-turnover pool. Most pork <sup>75</sup>Se was in the slow pool when dietary Se was adequate, but were in the fast pool when dietary Se was high. Increased urinary excretion with increased dietary Se has been reported in humans (39,40). In the present study, consumption of high-Se diets greatly increased urinary excretion of <sup>75</sup>Se from pork, and pork <sup>75</sup>Se tissue retention became more similar to broccoli. Increased dietary Se intake resulted in increased urinary <sup>75</sup>Se excretion, but the change in amount excreted was much greater for pork than broccoli. Rats fed high-Se diets excreted 47% more broccoli <sup>75</sup>Se into urine than rats fed adequate Se, but pork <sup>75</sup>Se urinary excretion was 300% greater for those fed high-Se diets.

Less <sup>75</sup>Se from broccoli than pork was retained in tissues of rats fed adequate dietary Se. In many tissues, more than twice as much <sup>75</sup>Se from pork, as compared to broccoli, was retained, and the <sup>75</sup>Se from broccoli was more labile than pork <sup>75</sup>Se. In tissues such as prostate, brain, and liver, 50–60% of the broccoli <sup>75</sup>Se present on d 2 had disappeared by d 10. However, the <sup>75</sup>Se from pork remained relatively stable from d 2 to d 10. Similar to whole-body kinetics, there were few differences between retention of <sup>75</sup>Se from pork or broccoli in the tissues of rats that were fed the high-Se diet.

Although specific chemical form(s) of Se in the radiolabeled foods were not identified, previous studies allow a good estimation of the forms that were probably present. Selenite incorporates into selenoprotein as SeCys and does not form SeMet; therefore, the majority of pork <sup>75</sup>Se was probably protein-bound SeCys. Many Se compounds have been reported to be present in broccoli, including selenoamino acids, but methylated



compounds, especially SeMSC, are most prevalent (41,42). Selenium from SeCys can be metabolized enzymatically to the selenide and excreted into urine or become available for selenoprotein synthesis, explaining the abundant labeling of selenoproteins by pork  $^{75}\text{Se}$  as well as the reported high bioavailability of beef Se when assessed by repletion of selenoprotein activity (15,27). Conversely, putative demethylation enzymes (43) must act on SeMSC to form selenide and make it available for protein synthesis. The demethylation pathway might be rate limiting, resulting in more excretion into urine and little selenoprotein production. Ultimately, complete understanding of the metabolism of Se from food sources depends on a more complete characterization of the chemical forms.

Selenium from broccoli did not efficiently incorporate into selenoproteins. It was previously reported that broccoli Se was less effective than selenite, selenate, or SeMet for restoration of GSH-Px activity in Se-depleted rats (21). Rats with chemically induced aberrant crypts were fed 0.1 or 1.0  $\mu\text{g Se/g}$  diet as selenate or high-Se broccoli and liver GSH-Px activity in rats fed 1.0  $\mu\text{g Se/g}$  was not affected by the dietary source of Se. However, GSH-Px activity in rats fed 0.1  $\mu\text{g Se/g}$  diet as selenate averaged 870 EU/mg protein, but it was significantly lower in rats fed Se from broccoli and rats averaged 126 EU mg/protein (16). GSH-Px activities were not affected by the Se source in the present study, but only two rats from each group were killed to determine Se status, therefore the experimental design did not allow comparison of enzyme activities and tissue Se concentrations.

High-Se broccoli and beef can be produced in high-Se regions of the world and both can accumulate sufficient concentrations of Se to make them effective dietary Se supplements. Broccoli that is used for phytoremediation of high-Se soils in California (44) produces an edible inflorescence that is enriched in Se (unreported data). The Se content of beef is moderate in most areas of the country but can be much higher when animals have access to high-Se forage (31,45). A 4-oz serving of beef from a high-Se area (such as central North or South Dakota) can provide more than 200  $\mu\text{g}$  of Se (3), the amount proven efficacious for cancer reduction. The present study demonstrates differences in Se metabolism between each of these foods, and these differences in metabolism are consistent with previously reported differences in bioavailability when Se is consumed at the nutritionally adequate level. The present study also suggests that the retention and distribution of Se from meat and broccoli are more similar when Se is fed at the supranutritional level.

## ACKNOWLEDGMENTS

The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

## REFERENCES

1. B. Pence, Dietary selenium and anti-oxidant status: toxic effects of 1,2-dimethylhydrazine in rats, *J. Nutr.* **121**, 138–144 (1991).
2. M. Beck, P. Kolbeck, L. Rohr, et al., Benign human enterovirus becomes virulent in selenium-deficient mice, *J. Med. Virol.* **43**, 166–170 (1995).
3. J. Clark, G. Combs, B. Turnbull, et al., Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin, *JAMA* **276**, 1957–1963 (1996).
4. Y. Xia, X. Zhao, L. Zhu, et al., Metabolism of selenate and selenomethionine by a selenium-deficient population of men in China, *J. Nutr. Biochem.* **3**, 202–210 (1992).
5. M. Beilstein and P. Whanger, Chemical forms of selenium in rat tissues after administration of selenite or selenomethionine, *J. Nutr.* **116**, 1711–1719 (1986).
6. J. A. Butler, M. A. Beilstein, and P. D. Whanger, Influence of dietary methionine on the metabolism of selenomethionine in rats, *J. Nutr.* **119**, 1001–1009 (1989).
7. H. E. Ganther and J. R. Lawrence, Chemical transformations of selenium in living organisms. Improved forms of selenium for cancer prevention, *Tetrahedron* **53**, 12,299–12,310 (1997).
8. H. E. Ganther, Pathways of selenium metabolism including respiratory excretory products, *J. Am. Coll. Toxicol.* **5**, 1–5 (1986).
9. R. Burk, Molecular biology of selenium with implications for its metabolism, *FASEB J.* **5**, 2274–2271 (1991).
10. C. Ip and H. E. Ganther, Activity of methylated forms of selenium in cancer prevention, *Cancer Res.* **50**, 1206–1211 (1990).
11. J. R. Hunt, Tailoring advice on dietary supplements: an opportunity for dietetics professionals, *J. Am. Diet. Assoc.* **102**, 1754–1755 (2002).
12. H. Meltzer, K. Bibow, I. Paulsen, et al., Different bioavailability in humans of wheat and fish selenium as measured by blood platelet response to increased dietary Se, *Biol. Trace Element Res.* **36**, 229–241 (1993).
13. H. Van der Torre, W. Van Dokkum, G. Schaafsma, et al., Effect of various levels of selenium in wheat and meat on blood Se status indices and on Se balance in Dutch men, *Br. J. Nutr.* **65**, 69–80 (1991).
14. H. M. Meltzer, G. Norheim, K. Bibow, et al., The form of selenium determines the response to supplementation in a selenium replete population, *Eur. J. Clin. Nutr.* **44**, 435–446 (1990).
15. B. Shi and J. Spallholz, Bioavailability of selenium from raw and cooked ground beef assessed in selenium-deficient Fischer rats, *J. Am. Coll. Nutr.* **13**, 95–101 (1994).
16. J. W. Finley, C. Davis, and Y. Feng, Selenium from high-selenium broccoli protects rats from colon cancer, *J. Nutr.* **130**, 2384–2389 (2000).
17. C. Ip, D. J. Lisk, and G. S. Stoewsand, Mammary cancer prevention by regular garlic and selenium-enriched garlic, *Nutr. Cancer* **17**, 279–286 (1992).
18. C. Ip and D. Lisk, Characterization of tissue selenium profiles and anticarcinogenic responses in rats fed natural sources of selenium-rich products, *Carcinogenesis* **15**, 573–576 (1994).
19. X.-J. Cai, E. Block, P. C. Uden, et al., *Allium* chemistry: identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas chromatography with atomic emission detection, *J. Agric. Food Chem.* **43**, 1754–1757 (1995).

20. P. D. Whanger, Selenocompounds in plants and animals and their biological significance, *J. Am. Coll. Nutr.* **21**, 223–232 (2002).
21. J. Finley, Selenium from broccoli is metabolized differently than Se from selenite, selenate or selenomethionine, *J. Agric. Food Chem.* **46**, 3702–3707 (1998).
22. J. W. Finley, C. Ip, D. Lisk, et al., Cancer-protective properties of high-selenium broccoli, *J. Agric. Food Chem.* **49**, 2679–2683 (2001).
23. C. D. Davis, H. Zeng, and J. W. Finley, Selenium-enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice, *J. Nutr.* **132**, 307–309 (2002).
24. J. W. Finley and C. D. Davis, Selenium from high-selenium broccoli is utilized differently than selenite, selenate and selenomethionine, but is more effective in inhibiting colon carcinogenesis, *BioFactors* **14**, 196 (2001).
25. J. Holden, R. Gebhardt, C. Davis, et al., A nationwide study of the selenium content and variability in white bread, *J. Food Comp. Anal.* **4**, 183–195 (1991).
26. J. B. van Ryssen, J. T. Deagen, M. Beilstein, et al., Comparative metabolism of organic and inorganic selenium by sheep, *J. Agric. Food Chem.* **37**, 1358–1363 (1989).
27. B. Shi and J. Spallholz, Selenium from beef is highly bioavailable as assessed by liver glutathione peroxidase (EC 1.11.1.9) activity and tissue selenium, *Br. J. Nutr.* **72**, 873–881 (1994).
28. K. J. Hintze, G. P. Lardy, and J. W. Finley, Selenium accumulation in beef: effect of dietary selenium and geographical area of animal origin, *J. Agric. Food Chem.* **50**, 3938–3942 (2001).
29. M. Grusak and S. Pezeshigi, Uniformly  $^{15}\text{N}$ -labeled soybean seeds produced for use in human and animal nutrition studies: description of a recirculating hydroponic growth system and whole plant nutrient and environmental requirements, *J. Sci. Food Agric.* **64**, 223–230 (1994).
30. J. W. Finley and C. D. Davis, Manganese absorption and retention in rats is affected by the type of dietary fat, *Biol. Trace Element Res.* **82**, 143–158 (2001).
31. J. Finley, L. Matthys, T. Shuler, et al., Selenium content of foods purchased in North Dakota, *Nutr. Res.* **16**, 723–728 (1996).
32. J. W. Finley, Manganese absorption and retention by young women is associated with serum ferritin concentration, *Am. J. Clin. Nutr.* **70**, 37–43 (1999).
33. Z. Kuzina, L. Genci, J. Kuruc, et al., Porovnanie aminokyselinoveho zlozenia hovadzieho a bravcoveho masa [Comparison of amino acid composition of beef and pork], *Acta Zootech.* **31**, 85–122 (1976).
34. S. Gerrior, Estimating nutrient contributions from lean beef and pork in the U.S. food supply series, *Fam. Econ. Nutr. Rev.* **9**, 38–43 (1996).
35. X. Wang and C. M. Parsons, Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals, *Poult. Sci.* **77**, 834–841 (1998).
36. C. E. Bodwell and B. A. Anderson, Nutritional composition and value of meat and meat products, in *Muscle as Food*, P. J. Bechtel, ed., Academic, Orlando, FL, pp. 321–369 (1986).
37. H. Y. Wen, R. L. Davis, B. Shi, et al., Bioavailability of selenium from veal, chicken, beef, pork, lamb, flounder, tuna, selenomethionine, and sodium selenite assessed in selenium-deficient rats, *Biol. Trace Element Res.* **58**, 43–53 (1997).
38. R. Martin, V. Young, J. Blumberg, et al., Ascorbic acid–selenite interactions in humans studied with an oral dose of  $^{74}\text{SeO}_3^{2-}$ , *Am. J. Clin. Nutr.* **49**, 862–869 (1989).
39. M. Robinson, H. Rea, G. Friend, et al., On supplementing the selenium intake of New Zealanders: 2. Prolonged metabolic experiments with daily supplements of selenomethionine, selenite and fish, *Br. J. Nutr.* **39**, 589–600 (1978).
40. J. Robinson, M. Robinson, O. Levander, et al., Urinary excretion of selenium by New Zealand and North American human subjects on differing intakes, *Am. J. Clin. Nutr.* **41**, 1023–1031 (1985).

41. M. T. Roberge, A. J. Borgerding, and J. W. Finley, Choice of extracting conditions in the speciation of compounds from high selenium broccoli will affect analytical results, *J. Agric. Food Chem.* **51**, 4191–4197 (2003).
42. X.-J. Cai, E. Block, P. C. Uden, et al., *Allium* chemistry: identification of natural abundance organoselenium compounds in human breath after ingestion of garlic using gas chromatography with atomic emission detection, *J. Agric. Food Chem.* **43**, 1751–1753 (1995).
43. Z. Zhu, W. Jiang, H. E. Ganther, et al., Activity of Se-allylselenocysteine in the presence of methionine gamma- lyase on cell growth, DNA integrity, apoptosis, and cell-cycle regulatory molecules, *Mol. Carcinog.* **29**, 191–197 (2000).
44. G. Banuelos, H. Ajwa, L. Wu, et al., Selenium-induced growth reduction in Brassica land races considered for phytoremediation, *Ecotoxicol. Environ. Safety* **36**, 282–287. (1997).
45. K. J. Hintze, G. P. Lardy, M. Marchello, et al., Areas with high concentrations of selenium in the soil and forage produce beef with enhanced concentrations of selenium, *J. Agric. Food Chem.* **49**, 1062–1067 (2001).
46. C. Ip and H. Ganther, Activity of methylated forms of selenium in cancer prevention, *Cancer Res.* **50**, 1206–1211 (1996).